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Cytokinins, growth, flower and fruit formation in *Vitis vinifera*

by

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Cytokinine, Wachstum, Blüten- und Fruchtbildung bei *Vitis vinifera*

Zusammenfassung. — Es wurden Untersuchungen über den Gehalt und die Änderungen der endogenen Cytokinine in den Wurzeln, Knospen, Trieben, Blättern, Blüten und Trauben von *Vitis vinifera* während des Austriebes, vor der Blüte, während der Vollblüte, des Beerenwachstums bis auf eine Größe von 8—12 mm, während der Traubenreife, des Blattfalles und der relativen Ruhe durchgeführt. Hierbei wurden Pflanzen verwendet, die sich in ihrem Wachstum sowie der Blüten- und Fruchtbildung unterschieden. Zu diesem Zweck wurde ein Teil der Reben vor dem Austrieb mit Chlorcholinchlorid (CCC) behandelt, wodurch das Längenwachstum um 12,6% verringert, die Anzahl der Blüten dagegen um 37,9%, die der Trauben um 39,2% und der Ertrag um 34,1% erhöht wurden.

Die Pflanzen mit gehemmtem Längenwachstum und geförderter Blüten- und Fruchtbildung enthielten im Durchschnitt mehr Cytokinine als die Kontrollpflanzen, u. z.: Wurzeln 10,2%, Knospen 17,3%, Triebe 34,2%, Blätter 9,8%, Blüten und Trauben 22,0%. Auch in Samen, Fleisch und Haut reifer Beeren wurden unterschiedliche Cytokininkonzentrationen festgestellt.

Introduction

Natural cytokinins have been found to be stimulants of cell division (18), but their role and importance are not restricted only to the process of cytokinesis. Together with auxins they play a role in the regulation of the cell growth and the differentiation (6, 7, 11, 24), the growth and the organogenesis (3, 24), the ageing, rest (19, 22), and several other processes in the plants (14).

According to CHAILAKHYAN *et al.* (5) and CHAILAKHYAN (4), nucleic acids metabolites, and in particular some purine bases, forming part of the composition of natural cytokinins, together with the auxins, play an important part in the metabolite preparation of the plants for blooming, by exerting a regulating influence on the quantitative aspect of this process.

So far we have no knowledge of investigations of the changes in the cytokinins in plants in relation to their flower and fruit formation. Considering the great importance of these processes in the vine, we set ourselves the task of investigating the changes in the cytokinins in various organs of the latter and during different moments and phases of its development, using plants with different growth, flower and fruit formation.

Materials and Methods

The investigations were carried out on the early table grape variety of Cardinal, grafted on a *Vitis berlandieri* × *V. riparia* Kober 5 BB rootstock. 5-year-old vines growing in the field were used.

In order to obtain plants differing in growth and flower and fruit formation, we treated the canes of half of the vines, prior to bud burst, with 9 per cent water solution of CCC (chlorocholine chloride), which inhibits the growth of the shoots but intensifies the flower and fruit formation (16, 17). In this connection, observations were carried out on the shoot growth, the number of inflorescences and bunches, and also on the yield of grapes both for the CCC-treated and non-treated vines, the latter as control tests.

The investigations of the cytokinins were carried out in the roots, the buds of the fruit canes and in the shoots, leaves, inflorescences and clusters, that had grown from the same canes. Samples were taken during bud burst (a), prior to anthesis (b), during full bloom (c), growth of the berries (d), ripening of the grapes (e), during leaf-fall (f) and the period of relative rest (g).

The cytokinin content of the roots was studied in their absorbing and conducting parts, in the buds of the nodes 5—7 and 10—12 of the canes at the time of bud burst, in the shoots from the internodes 6—8, 12—14, and 18—20, and in the leaves from the nodes of these storeys. Its determination in the inflorescences was carried out before anthesis and during full bloom, and in the clusters, when the berries reached the size of a bean (8—12 mm), and during ripening.

The extraction of the cytokinins from the various organs was done with the aid of cooled, freshly distilled methanol, according to NITSCH and NITSCH (20) and JAKO (13).

Because of the strong pigmentation of the leaves, it became imperative to separate the purine bases by precipitating with silver nitrate. The methanol extracts from the other organs, as our investigations showed, did not require any additional refining.

The separation of the cytokinins was done with the aid of Whatman 3MM chromatographic paper and solvent ethyl-acetate, formic acid and water in a 10.2 : 1.0 : 5.8 ratio. As a biotest for cytokinin activity, germinants of *Amaranthus caudatus* L. were used.

The cytokinin amount was determined by the beta-cyanine pigment formed in the biotest, by means of spectrophotometry and corresponding calculation of a standard curve for various kinetin concentrations, which had been prepared in advance.

Results and Discussion

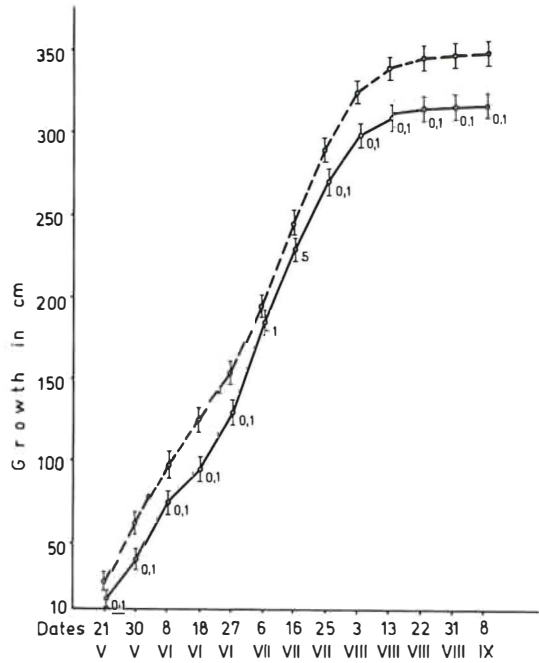
The treatment of the vines with CCC prior to bud burst provoked inhibition of the shoots throughout the vegetation period (Fig. 1). The greatest differences between the two variants were observed during the period of the most intensive growth (in June), at the time immediately before anthesis and during full bloom. In July, these differences decreased, but in August they again increased, owing to the earlier ending of the growth of the CCC-treated vines.

CCC-treatment caused the formation of more inflorescences (an increase of 37.9 per cent), a larger number of clusters (39.2 per cent more), and a higher yield of grapes (34.1 per cent more). The improved yield of these vines resulted from the more intensive formation of inflorescences and clusters, caused by the retardant (Fig. 2).

These results have shown that when treating part of the vines with CCC, plants with less intensive growth of shoots, but with a more intensive flower and

Fig. 1: Dynamics of the shoot growth. — = CCC treatment, - - - = Control. Bars indicate standard error of means at a significance level expressed by the added numbers.

Abb. 1: Dynamik des Triebwachstums. — = CCC-Behandlung, - - - = Kontrolle. Die Standardfehler bei den angegebenen Signifikanzschwellen (Zahlen) sind eingetragen.



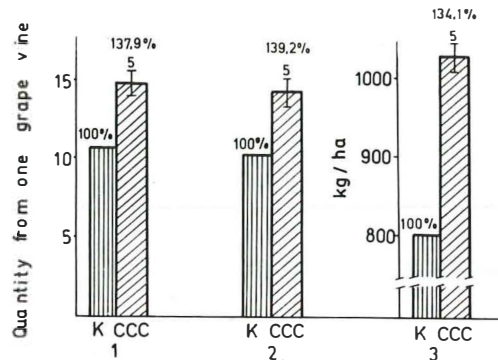
fruit formation, are obtained. In that way, suitable conditions were created for the envisaged investigation according to the present aim.

The cytokinin content in the roots is shown in Fig. 3. The absorbing roots contain a considerable amount of cytokinins during the beginning of the vegetation period, and it increases at the time of anthesis, but reaches its peak during berry growth. Subsequently, a gradual decrease is noted, which is particularly marked after the ripening of the grapes. The minimum cytokinin content is reached during the leaf-fall period, while during the period of relative rest the cytokinin activity in the absorbing roots has increased again.

The changes in the cytokinins in the absorbing roots are almost uniform with those in the conducting roots, which indicates that the rhythm of cytokinin bio-

Fig. 2: Number of inflorescences (1), clusters (2), and yield (3). K = Control, CCC = CCC treatment. Further explanations see Fig. 1.

Anzahl der Blüten (1) und der Trauben (2) sowie Ertrag (3). K = Kontrolle, CCC = CCC-Behandlung. Weitere Erläuterungen s. Abb. 1.



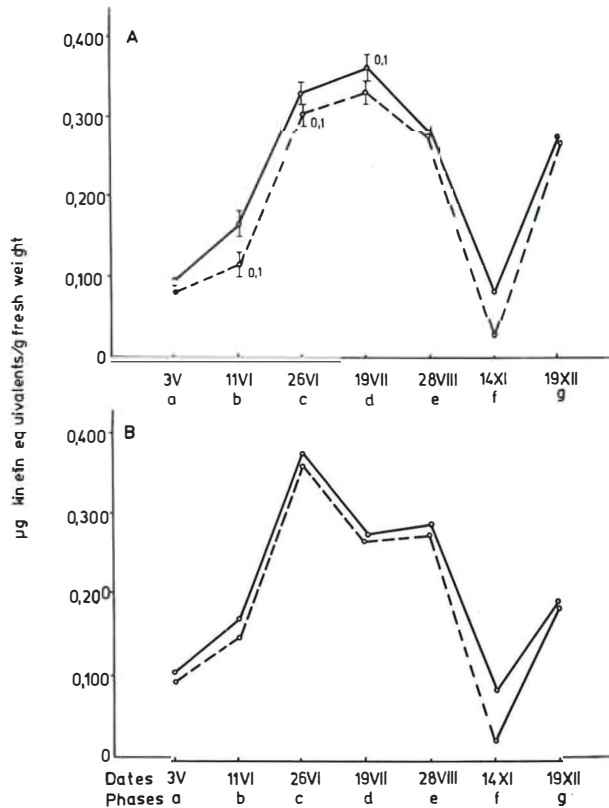


Fig. 3: Cytokinin content in the roots. A) Absorbing roots, B) conducting roots. Further explanations see Fig. 1.

Cytokiningehalt in den Wurzeln. A) Absorbierende Wurzeln, B) leitende Wurzeln. Weitere Erläuterungen s. Abb. 1.

synthesis in the former corresponds to their movement through the conducting part of the root system.

The plants with inhibited growth but with intensified flower formation contain more cytokinins than the control plants, both in the absorbing and the conducting roots. During the time of bud burst, the differences between the two variants are small, 0.010 $\mu\text{g/g}$ fresh material for the absorbing, and 0.012 $\mu\text{g/g}$, for the conducting roots. At the beginning of bloom, these differences increase, the treated plants contain more cytokinins, both in the absorbing and in the conducting parts of the root system, but the increase is greater in the former (0.049 $\mu\text{g/g}$) than in the latter (0.020 $\mu\text{g/g}$). Differences in the cytokinin level of the two variants are observed also during the subsequent phases. It is only during the relative rest that their cytokinin content becomes almost the same.

The most likely explanation for the resulting differences in the cytokinin content in the roots of the two kinds of plants is that because of the different growth of their shoots, the expending of the cytokinins, synthesized in the roots, is not the same in the processes of growth throughout the vegetation period. For this reason, the cytokinin content remains higher in the roots of the treated plants. The insigni-

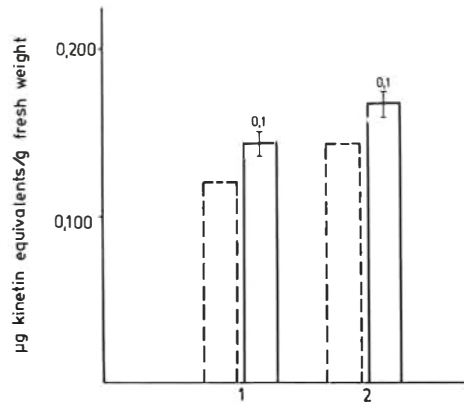


Fig. 4: Cytokinin content in the buds. 1) Nodes 5—7, 2) nodes 10—12. Further explanations see Fig 1.

Cytokiningehalt in den Knospen. 1) Knoten 5—7, 2) Knoten 10—12. Weitere Erläuterungen s. Abb. 1.

ficant difference in the cytokinin activity in the two variants during the relative rest period is probably due to the lesser growth activity in both, owing to the already subsided active bioprocesses also in the shoots.

The bursting buds contain considerable amounts of cytokinins (Fig. 4). When comparing them from both storeys, it is noticeable that those of the higher part of the fruit cane contain more cytokinins. This is probably due to the fact that the upper buds develop earlier than those in the lower part of the canes, because it is well-known (8) that bursting buds contain more cytokinins compared with those at rest, or those which are less advanced in the intervening of that state.

The important amount of cytokinins found in our experiments corresponds also to the experimental results of other authors in respect of other plants, who have found an increased level of these phytohormones in the buds when interrupting the rest (10, 21).

When comparing the cytokinin content in the control and the treated plants, it is interesting to note that in the buds of the latter it is higher.

The investigations of the endogenous cytokinins in the shoots from the different storeys (Fig. 5) have shown that in the lowest internodes — 6—8 — initially, before anthesis, the cytokinin content is low, but afterwards it increases considerably and reaches its maximum during the growth of the berries. The period after the growth of the berries is characterized by a decrease in the cytokinin content, which intensifies during leaf-fall, when the lowest content is found. During the relative rest, the cytokinin activity of the shoots again increases. The internodes 12—14 ad 18—20 appear later, but here also, the changes in the cytokinin content follow the same line during the corresponding moments and phases.

A comparison of the cytokinin content of the various storeys of the vine shoots at one and the same time or stage shows that it is at its lowest in the highest situated internodes, and at its highest, in the lowest ones — 6—8. This is probably due to the greater remoteness of that storey from the root system and to the lesser mobility of cytokinins compared with the other growth regulators.

In the vines with inhibited growth and intensified flower and fruit formation, throughout the vegetation period, with the exception of the leaf-fall phase, the cytokinin content was higher. With the intensification of the processes of growth, the differences in the cytokinin level in both variants increase, in favour of the treated plants anyway (Fig. 5). The differences are particularly important in the two lower storeys. At the time of full bloom they amount to 0.088 µg/g for the inter-

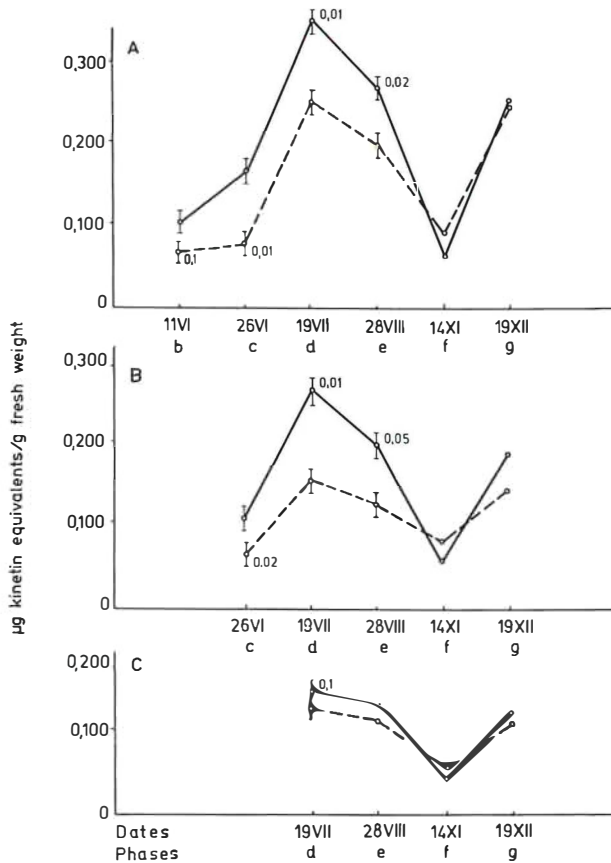


Fig. 5: Cytokinin content in the shoots. A) Internodes 6—8, B) internodes 12—14, C) internodes 18—20. Further explanations see Fig. 1.

Cytokiningehalt in den Trieben. A) Internodien 6—8, B) Internodien 12—14, C) Internodien 18—20. Weitere Erläuterungen s. Abb. 1.

nodes 6—8, and $0.043 \mu\text{g/g}$ for the internodes 12—14, and they increase further during the ripening period to $0.100 \mu\text{g/g}$ and $0.130 \mu\text{g/g}$, respectively. During the remaining phases, which are characterized by the discontinuation of the shoot growth, the quantitative differences between the two variants decrease quite noticeably, and in the leaf-fall phase, there are less cytokinins in the shoots of the treated vines. The decrease in the differences between the control plant and the treated vines is most likely due to the subsided growth processes in the shoots in connection with the preparation of the plants for the period of relative rest.

As was found for the roots, in plants with a different growth and different flower and fruit formation, the character of the changes in the cytokinin-type phytohormones in the shoots remains similar throughout the various phases.

When comparing the cytokinin content in the roots and the shoots (Figs. 3, 5), it is seen that during the vegetation period and the relative rest period the root system is richer in such phytohormones. Besides that, the changes in the amount of cytokinins during all investigated phases are uniform for both organs. This means

that the rhythm of their changes in the shoots corresponds to the rhythm of their biosynthesis and transport from the roots. In other words, a close positive dependence exists between the biosynthesis of cytokinins in the roots and their level in the shoots.

A similar close positive dependence is observed also between the changes in the cytokinins in the roots and the shoots on the one hand, and the growth of the shoots themselves on the other. With intensified cytokinin synthesis in the root system and their transport to the shoots, the latter's growth is also intensified, the maximum content coinciding with the most active growth, and the minimum with the complete cessation of the processes of growth.

It is interesting to note that after reaching a minimum during the period of the leaf-fall, during the time of relative rest the cytokinin content of the roots and shoots again begins to go up. This fact may be explained by the physiological peculiarities of the plant during that period.

The investigations of BABRIKOV and BRAIKOV (1) on the root system of the Bolgar strain grafted on a *V. berlandieri* × *V. riparia* Kober 5 BB rootstock, i.e. on the same rootstock on which the Cardinal strain is also grafted, have shown that after a certain lagging in the growth of the absorbing roots in the month of November, i.e. of the roots in which the basic biosynthesis of the cytokinins takes place, during the subsequent period of relative rest (i.e. in December, January and February), their growth is again intensified, although the average daily temperature of the soil had been below 0 °C in January and only 2–4 °C in February. This shows that during the period of relative rest, side by side with the growth in the root system, the synthesis of cytokinins probably also goes up and through the conducting roots, the cytokinins are transported to the shoots. But as during that same period the shoots still do not grow and no consumption of cytokinins takes place, not only does the cytokinin content begin to increase in the shoots, but also in the roots. This conception is also confirmed, according to us, by the fact that the greatest amount of cytokinins is found during that period in the absorbing roots, followed by the conducting roots, and finally the shoots, as well as by the circumstance that the further the internodes are from the roots, the weaker is found to be the increase in the cytokinin content during the relative rest period.

The content of cytokinin and its changes as regards the leaves, are shown in Fig. 6. Because of frostbite during the autumn, no investigations were carried out during the leaf-fall phase. The phase of full bloom is characterized by a relatively high level of cytokinin presence. The further progress of the vegetation is accompanied by a decrease in the cytokinin level. Most likely the decrease, which is found in the leaves at the time of berry growth, is due, as ENGELBRECHT (9) assumes for the poplar, the birch and the maple, to a certain dilution of these substances in the largely increased foliage at that time and to their inclusion in the highly active metabolism characterizing this stage.

At the time of the ripening of the grapes, the leaves' cytokinin activity has increased again. The increase in cytokinin content in ageing leaves which we have found is confirmed also by other authors for other plants (9, 12, 25), and they are inclined to explain this fact by an accumulation of cytokinins in the leaves after the cessation of the apical activity in the form of a cytokinin fraction of a nucleotide character, which is less active compared with the free bases and their ribosides. It is a form of preservation of the hormone and is not in a position to avert the destruction of the chlorophyll, i.e. the yellowing of the leaves. We also assume that

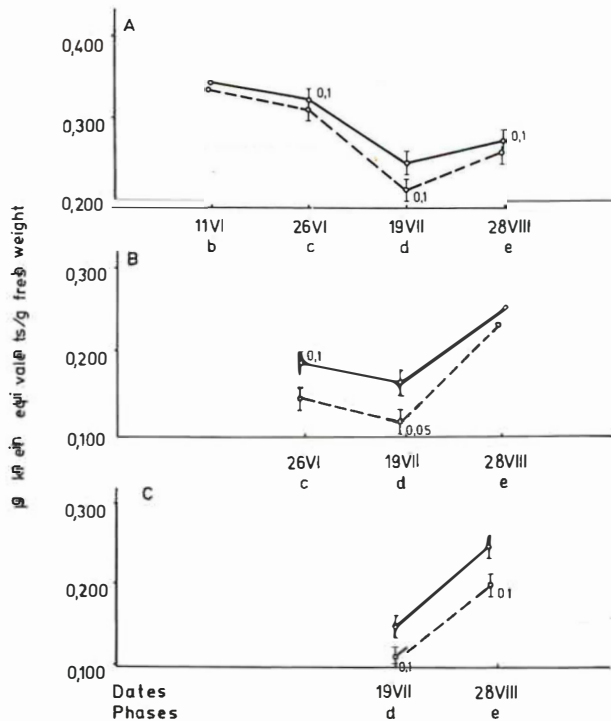


Fig. 6: Cytokinin content in the leaves. A) Nodes 6—8, B) nodes 12—14, C) nodes 18—20. Further explanations see Fig. 1.

Cytokiningehalt in den Blättern. A) Knoten 6—8, B) Knoten 12—14, C) Knoten 18—20. Weitere Erläuterungen s. Abb. 1.

the increased cytokinin content which we found at the end of the month of August is due to this nucleotide fraction, which is characteristic of ageing leaves.

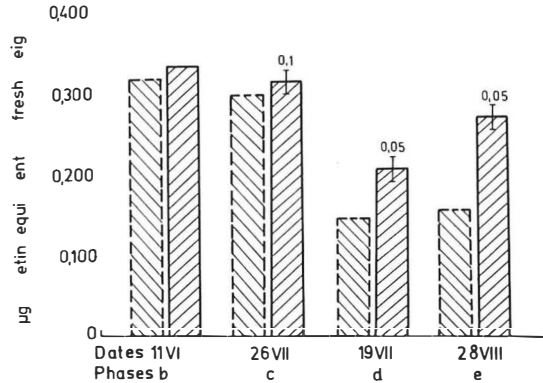
By the amount of cytokinins they contain, the leaves of the three storeys differ, irrespective of the circumstance that their change is uniform. The richest content in such growth regulators is found in the leaves of the nodes 6—8, and the poorest in the leaves of the nodes 18—20. This may be due to the same factors as with the shoots, i.e. to the different remoteness of the several storeys from the root system and to the minor mobility of cytokinins.

The quantitative differences of the cytokinins in the leaves of the two groups of plants at the beginning of anthesis were insignificant. During full bloom they went up to 0.013 $\mu\text{g/g}$ for the nodes 6—8, and to 0.042 $\mu\text{g/g}$ for the nodes 12—14. At the time of berry growth the differences amounted to 0.032 $\mu\text{g/g}$ for the lower storey, to 0.049 $\mu\text{g/g}$ for the middle storey, and 0.041 $\mu\text{g/g}$ for the upper storey. After the cessation of growth, at the time of ripening of the grapes, the differences between the leaves of vines with intensified flower formation decreased, but remained considerable. Here too, as in the roots and the shoots, the character of the change in the cytokinins during the separate phases does not change.

The amount of the cytokinins was also investigated in the inflorescences both before and after blooming, and in the unripe and ripe grapes (Fig. 7). It should be noted that the inflorescences are characterized by a higher cytokinin

Fig. 7: Cytokinin content in the inflorescences (I) and the clusters (II). Further explanations see Fig. 1.

Cytokiningehalt in den Blüten (I) und Trauben (II). Weitere Erläuterungen s. Abb. 1.



content. The younger and still growing inflorescences at the start of the bloom are richer in cytokinins. With the further growth in their development, at the time of full bloom, and of their turning into clusters, the cytokinin content continues to fall. But with the beginning of ripening, the cytokinin content has increased again, with a higher rate of increase for the CCC-treated vines.

A comparison of the cytokinin contents in the inflorescences and the roots shows that the organs of reproduction have a higher content of such growth substances. The high content of cytokinin-type phytohormones in the developing fruits and seeds has suggested to several authors the idea of examining these organs, side by side with the roots, as a place of quite active biosynthesis of cytokinins (2, 15).

According to SIRON (23), when the cytokinin supply from the roots decreases, a new centre of cytokinin biosynthesis is formed in the growing fruits. According to our results, if such a centre exists, it is not localized in the clusters, but in the inflorescences, because they show the highest cytokinin content. It is possible that this centre may later on continue to function in the growing berries and the ripening grapes as well, but with a lessened activity.

From Fig. 7 it may be seen also that the differences between the vines with inhibited growth and with intensified flower and fruit formation on the one hand, and the control plants on the other, both before and at the time of blooming, are not very important, but can be proved mathematically. The control plants have a lower cytokinin content. When the inflorescences begin to turn into bunches of grapes, the amount of cytokinin begins to decrease in both variants, but the differences between them become greater. Thus, for instance, at the time of berry growth, the clusters of treated vines contained 43.6 per cent more cytokinins than the clusters of the control plants, and this difference increased to 76 per cent during ripening.

A detailed investigation of the cytokinin activity of individual parts of ripe berries (Fig. 8) has shown that it is highest in the skin, followed by the seeds and the pulp.

A rather different distribution of the cytokinins was observed for the two groups of plants. The skins of the berries from treated vines were found to contain less cytokinins than those of the control plants, but it seems that at the expense of this fact, cytokinins were discovered also in the rachises of the same bunches of grapes, while in the rachises of the control vines only traces were found. The cytokinin content of the pulp of the berries of this variant and, in particular of the

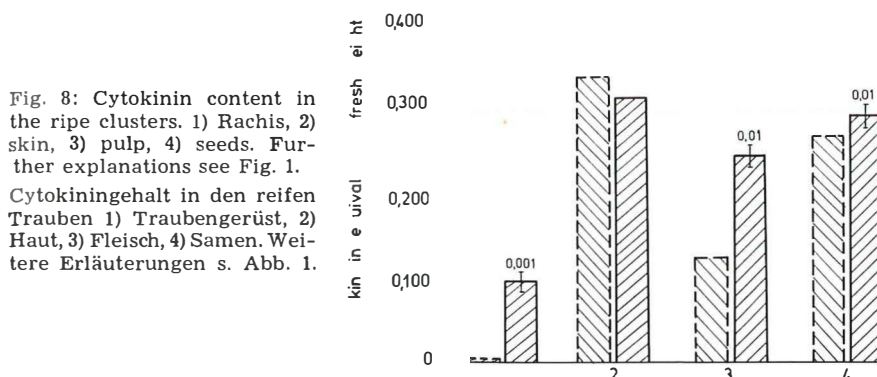


Fig. 8: Cytokinin content in the ripe clusters. 1) Rachis, 2) skin, 3) pulp, 4) seeds. Further explanations see Fig. 1.

Cytokiningehalt in den reifen Trauben 1) Traubengerüst, 2) Haut, 3) Fleisch, 4) Samen. Weitere Erläuterungen s. Abb. 1.

seeds, was quite high. The differences between the two variants in respect of the pulp were rather important compared to those in respect of the skins and the seeds. It is most likely that in the latter a certain limit of saturation with cytokinins is reached and that this limit is determined by the self-regulating biological mechanism of the plant, owing to which the differences between treated plants and control plants remained small.

Summary

Investigations were carried out on content and changes of the endogenous cytokinins in roots, buds, shoots, leaves, inflorescences and clusters of *Vitis vinifera* during bud burst, prior to anthesis, during full bloom, growth of the berries to 8–12 mm, ripening of the grapes, during leaf-fall and the period of relative rest.

In order to obtain plants, differing in growth, flower and fruit formation, half of the vines were treated with CCC prior to bud burst. Consequently, the growth of the treated plants was inhibited by 12.6%, the number of the inflorescences was increased by 37.9%, the number of the clusters by 39.2% and the yield of the grapes by 34.1%.

The plants showing inhibited growth and stimulated flower and fruit formation contain for all the phases investigated more cytokinins compared to the control: roots 0.021 $\mu\text{g/g}$ fresh material or 10.2%, buds 0.023 $\mu\text{g/g}$ or 17.3%, shoots 0.040 $\mu\text{g/g}$ or 34.2%, leaves 0.024 $\mu\text{g/g}$ or 9.8%, inflorescences and clusters 0.05 $\mu\text{g/g}$ or 22.0%.

Different amounts of cytokinins were established in the individual parts of ripe berries (skin, seeds and pulp).

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